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Research Article

Bioconversion of heptanal to heptanol by *Saccharomyces cerevisiae*

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Abstract

***Saccharomyces cerevisiae* is widely known for its catalytic activity on substrates such as aldehyde and ketone. Interestingly, the activity of *S. cerevisiae* on heptanal ($C_6H_{13}CHO$), in spite of its being a very common aldehyde, has not been explored. The main objective of this study was therefore to investigate the bioconversion of heptanal, using a strain of the yeast *S. cerevisiae*. Bioconversion parameters such as incubation period, pH, concentration of substrate, yeast and maltose were also optimized. The study revealed heptanol as the major product. The optimum conditions for biotransformation were found to be: 3 days incubation; pH 7.0; heptanal concentration 0.15 ml/100 ml medium; and *S. cerevisiae* concentration of 0.15 g/100 ml medium. Reduction in maltose content (to 0.3 g maltose/100 ml medium) showed increased conversion of heptanal. Heptanoic acid and 2-hydroxyheptanoic acid were obtained as two minor co-products. The overall study showed that *S. cerevisiae* converted heptanal to heptanol by a yield of 68.9 ± 1.1% w/w under optimum conditions.**

Keywords: **bioconversion; heptanal; heptanol; *Saccharomyces cerevisiae***

Introduction

In recent times, the chemo-enzymatic synthesis of organic molecules has acquired importance due to several attractive features, such as less vigorous reaction conditions, a single-step synthetic route, a highly specific reaction product (usually not attainable by a single step through conventional chemical process), and also a higher yield on many occasions. Study of enzymatic or bioconversion of aldehydes using *Saccharomyces cerevisiae* has been reported by a numbers of authors. Conversion of benzaldehyde and vanillin to their corresponding alcohols, by growing cells of *S. cerevisiae* in the presence of cyclic dextrins at the same molar concentration as the substrate, has been reported (Bar, 1989). Bioconversion of benzaldehyde to benzyl alcohol in aqueous–organic solvent biphasic systems by wild-type and mutant strains of *S. cerevisiae* has been investigated (Nikolova and Ward, 1991) and it was reported that the conversion rates in aqueous media were two to three times higher than in hexane containing 2% moisture. A comparison of the bioconversion of aromatic aldehyde (benzaldehyde) and selected substituted aromatic aldehydes (*O*-tolualdehyde and 1-chlorobenzaldehyde) to phenylacetyl carbinol, using sucrose and sodium pyruvate as co-substrates, has been studied (Long and Ward, 1989). Conversion of monoterpene aldehydes such as citral and (±) citronellal by *S. cerevisiae* has also been reported (Chatterjee *et al.*, 1999).

There were also reports on the bioconversion of compounds other than aldehydes using *S. cerevisiae*. Extractive bioconversion of 2-phenyl ethanol from L-phenylalanine has been done (Stark *et al.*, 2002). Co-culture of *S. diastaticus* and

S. cerevisiae 21 (24.8 g/l), and co-culture fermentation with *Endomycopsis capsularis* and *S. cerevisiae* 21 (16.0 g/l) and comparison with the monoculture of *S. diastaticus* and *E. capsularis* have been studied (Verma *et al*., 2000), with the intention of producing ethanol. Another study (Compagno *et al*., 1993) revealed that lactose–whey can be converted to fructose diphosphate (FDP) with recombinant *S. cerevisiae* cells, where whey can be used as the carbon source for the growth of *S. cerevisiae*. The bioconversion of 6,7-epoxygeraniol was studied (Anio and Huszcza, 2005) and it was shown that treatment with *S. cerevisiae, Candida parapsilosis* and *C. kefyr* reduced the substrate to 6,7-epoxycitronellol (30–33% of chloroform extracts) and with *Yarrowia lipolytica, Botrytis cinerea* and *S. cerevisiae* promoted the cyclization of 6,7-epoxygeraniol to 2-methyl-2-(2-hydroxyethyl)-5-(2-hydroxyprop-2-yl) tetrahydrofuran (11–99% of chloroform extracts). Conversion of ethanol and 2-butanol to ethanal and 2-butanal by immobilized cells of *S. cerevisiae* PTCC5080 have been investigated (Norouzian *et al*., 2003). Using *S. cerevisiae, Torulaspora delbrueckii* and *Kluyveromyces lactis* (King and Dickinson, 2000), monoterpene alcohols of significance in the alcoholic beverage industries could be bioconverted; according to these authors, *S. cerevisiae* and *K. lactis* reduced geraniol to citronellol. They also reported that these three yeasts have converted geraniol and nerol to linalool, and linalool and nerol to monocyclic α-terpineol.

Although bioconversion of several aldehydes has been attempted by different researchers, the same has not been investigated on heptanal, a very common perfumery raw material and a product of pyrolysis of castor oil, using *S. cerevisiae*. The present investigators have therefore studied the biotransformation of heptanal using *S. cerevisiae*, with the intention of searching the types of products formed, tyhe yield and the optimal reaction conditions.

Materials and methods

All the chemicals and solvents used in this study were of analytical reagent grade and were purchased from SD Fine Chem (Boisar, Mumbai, India). Heptanal (purity 80.87%) was a gift from Jayant Oils and Derivatives Ltd (Vadodara, India). Maltose, yeast extract and peptone (an enzymic hydrolysate of animal tissues) was supplied by Himedia Laboratories Pvt. Ltd (Mumbai, India).

Microorganism

Saccharomyces cerevisiae (dry baker's yeast), used in this study, was a product of United Trading Corporation (Mumbai, India) and was purchased from the local market and stored in a refrigerator at 5 °C.

Medium composition and conditions

Liquid medium used for the study of bioconversion contained maltose 5.0 g/l, yeast extract 0.5 g/l, peptone 5.0 g/l, KH_2PO_4 0.5 g/l, $MgSO_4$ 0.2 g/l. After dissolving all the ingredients, the pH was maintained at 5.2, the broth was distributed into Erlenmeyer flasks (capacity 250 ml, and each containing 100 ml), which were sterilized in an autoclave under standard conditions.

Optimization of incubation period

For optimization of the incubation period, in each of the flasks 0.15 g dry yeast and 0.15 ml heptanal was added aseptically. The resulting broths (containing organism and substrate) were divided into four sets of three flasks. Samples from each set were collected after 2, 3, 4 and 5 days, respectively, and analysed by thin-layer chromatography (TLC) and gas chromatography (GC) to ascertain whether any sort of transformation had taken place. Simultaneously, a control experiment was carried out without *S. cerevisiae* by adding substrate directly into the sterile broth.

Optimization of pH

After optimizing the incubation period, the pH of the medium was optimized for the highest conversion. This was done in an experiment designed as above. The flasks containing sterile growth medium were divided into five groups of three and in each group the pH was maintained at 4.0, 5.0, 6.0, 7.0 and 8.0, respectively.

Optimization of substrate concentration

For this, 0.15 g dry yeast was inoculated into each flask containing medium, pH 7.0, and in

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each of four groups of three flasks 0.10, 0.15, 0.20 and 0.25 ml heptanal was added under sterile conditions. Samples from each set were collected after 3 days and analysed as described above.

Optimization of dry yeast concentration

To optimize the yeast requirement for optimal conversion, flasks containing 100 ml sterile growth medium, pH 7.0, was taken and divided into four groups of three flasks. In each of the four groups, 0.10, 0.15, 0.20 and 0.25 g dry yeast was inoculated and 0.15 ml heptanal was added aseptically, and grown for 3 days. Finally the samples were analysed.

Effect of carbon source

Growth medium, pH 7.0 (the optimum) and containing varying amounts of maltose (0.1–0.5 g/100 ml medium) was prepared and sterilized. Then optimum amounts of heptanal and yeast were added and grown for 3 days, after which the product was recovered and analysed for conversion.

Extraction and purification of products

After treatment for a definite period (e.g. 3 days) the treated mass was extracted three times with chloroform (20 ml each time). The broth containing crude product was transferred to a separating funnel and, after adding chloroform, it was shaken gently and the two layers (aqueous and organic) were allowed to separate. The organic phase was dried by passing it through anhydrous $NaSO_4$ and was then evaporated by gently heating over a waterbath at 80 °C to obtain the crude product.

The product so obtained was subjected to TLC on silica gel G-coated glass plate (0.5 mm thick). The product was applied in band form and the plate was developed using the solvent system hexane : diethyl ether, 80 : 20 v/v. After development the bands were visualized in iodine vapour. The product band was marked, and scooped out from the plate. The silica gel (containing the product) was then washed with chloroform until free from product. The washings were then evaporated on a water bath under vacuum and analysed by GC–mass spectrometry (GC–MS), Fourier Transform Infrared spectrometry (FTIR) and Nuclear Magnetic Resonance (NMR) spectrometry.

Analytical methods

The reaction products were analysed by TLC on glass plates (20 × 20 cm) coated with a 0.5 mm layer of silica gel G. The plate was developed in a solvent system (of 100 ml) composed of hexane : diethyl ether (80 : 20 v/v) and the spots were identified by iodine absorption. No transformation was found in the control sets, as evidenced by TLC.

Chemical structures of the biotransformed product were established by FTIR, NMR and GC–MS spectroscopy. FTIR spectra were obtained using a Perkin-Elmer Spectrum-GX spectrophotometer on a KBr disk. Proton NMR spectra in $CDCl_3$ were obtained using a Bruker Advance spectrophotometer at a frequency of 400 MHz. GC–MS spectra of the compound were obtained using a Perkin-Elmer Autosystem XL gas chromatograph and turbo mass–mass spectrometer, equipped with a PE-5 MS column. Helium was used as the carrier gas at a flow rate of 1 ml/min, Injection port and detector port temperatures were maintained at 220 °C and 250 °C. Oven temperature was programmed as follows: initial temperature, 60 °C; final temperature, 250 °C; rise in temperature, 10 °C/min.

Results and discussion

A scheme of the bioconversion reaction with the raw material used and products formed is shown in Figure 1. The three products formed through the microbial treatment of heptanal have been named P1, P2 and P3.

Identification of bioconversion products

The mass spectrum of product P1 was similar to that of heptanol. The intense fragment ion peaks

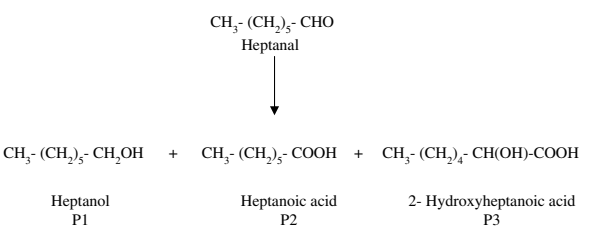

Figure 1. Scheme of the bioconversion reaction using *S. cerevisiae*

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were observed at *m/z* 83, 70, 56, 55, 43, 41 and 31 (relative intensity 9%, 99%, 97%, 89%, 73%, 89% and 34%, respectively). The important peak at *m/z* 70 was obtained due to McLafferty rearrangement. The cleavage of methyl ion ($-CH_3$) from *m/z* 70 gave a strong peak at *m/z* 55. Peaks at *m/z* 41 and 56 were obtained due to the cleavage of ethyl ($-CH_3CH_2$) and methylene ($-CH_2$) ions from *m/z* 70. The peak at *m/z* 31 was obtained due to $(CH_2{=}OH)^+$ ions. A primary alcohol also gave a peak at *m/z* 31. The loss of water molecules from the molecular ion peak (*m/z* 116) gave a small peak at *m/z* 98.

The mass spectrum of product P2 resembled that of heptanoic acid. The spectrum showed fragment ion peaks at *m/z* 87, 73, 60, 45 and 43 (relative intensity, 16%, 46%, 100%, 13% and 32%, respectively). The spectrum of P2 showed a strong peak at *m/z* 60 due to formation of $[CH_2{=}C(OH)_2]^+$ ions, as a result of McLafferty rearrangement. Formation of $[CH_2{=}CH{-}C(OH)_2]^+$ ions gave a peak at *m/z* 73. The peaks at *m/z* 43 and 87 correspond to $[CH_3(CH_2)_2]^+$ and $[CH_2{=}CH{-}CH_2{-}C(OH)_2]^+$ ions, respectively. The formation of carboxylate ions $(COOH)^+$ gave a peak at *m/z* 45.

The mass spectrum of product P3 indicated it to be 2-hydroxyheptanoic acid. The spectrum showed fragment ion peaks at *m/z* 113, 57, 55, 45, 43, 41 and 39 (relative intensity, 23%, 22%, 79%, 28%, 100%, 27% and 9%, respectively). The most intense band was at *m/z* 43. This was generally a peak due to $(C_3H_7)^+$ ions (Silverstein and Webster, 2007). The loss of two hydrogen atoms successively from *m/z* 43 led to the formation of ion peaks at *m/z* 41 and 39. The peak at *m/z* 45 was due to $(COOH)^+$ ions. The formation of $(CH_3{-}CH_2{-}CH_2{-}CH_2)^+$ ions gave a peak at *m/z* 57, from which the removal of two hydrogen atoms led the formation of an ion peak at *m/z* 55. An ion peak at *m/z* 113 was attributed to $[CH{=}CH{-}CH{=}C(OH){-}COOH]^+$.

IR spectra of products (mixtures) obtained from the microbial treatment of heptanal showed bands at 3350, 2930, 2858, 1713, 1467, 1378 and 1057 cm^{-1}. The peak at 1713 cm^{-1} may be assigned to C=O stretching of carboxylic acid where the carboxylate group was not ionized (Dyer, 1987). A broad band at 3350 cm^{-1} was ascribed to –OH stretching of the carboxylate moiety. The –OH stretching mode of alcohol was also absorbed in the region. The spectra showed peaks at 2930 and 2858 cm^{-1} due to C–H stretching, indicating the presence of alkyl groups. A strong peak at 1057 cm^{-1} was due to the C–O stretching mode. The strong absorption at 1467 cm^{-1} was attributed to $-CH_2$ bending and/or asymmetric bending of the $-CH_3$ moiety. The moderately strong absorption at 1379 cm^{-1} was assigned to the umbrella bending of $-CH_3$ groups.

The ^{1}H-NMR spectrum (in $CDCl_3$) of the product mixture consisted of a triplet in the region 3.6–3.7 ppm, each of which was split into doublets. This may be ascribed to the methylene (CH_2) proton of the alcohol moiety. Each of these splits into doublets due to coupling with hydroxyl (–OH) proton. The triplet at 0.9 ppm was due to methyl ($-CH_3$) group attached to a methylene ($-CH_2$) group. The peak at 2.2 ppm was possibly due to the carboxylic proton. The resonances in the range 1.0–2.0 ppm were due to $-CH_2$ protons and it is likely that the –OH protons of alcohol groups also had resonance in this region only (Silverstein and Webster, 2007; Namikoshi *et al*., 2002).

The mass spectra of the samples P1, P2 and P3 suggest these to be heptanol, heptanoic acid and 2-hydroxyheptanoic acid, respectively. The IR and NMR data also supported this to a large extent.

Effect of incubation period

Figure 2 shows the effect of incubation period on the bioconversion of heptanal and also the products obtained. It can be seen that after 3 days of incubation, residual heptanal content ($0.2 \pm 0.05\%$ w/w) was minimal and the major product (heptanol) content was maximal ($67.3 \pm 1.13\%$ w/w) in the product mixture. It was also found that the heptanol content in the product mixture decreased from $67.3 \pm 1.13\%$ to 63.7 ± 0.85–$57.0 \pm 1.2\%$ w/w after 3 days of incubation. However, the heptanoic acid content ($1.8 \pm 0.35\%$ w/w) was found to be least after 3 days of incubation, whereas it was highest ($6.2 \pm 0.3\%$ w/w) after 4 days of incubation. The content of the third product, 2-hydroxyheptanoic acid ($5.7 \pm 0.4\%$ w/w), was also found to be highest after 4 days of incubation.

Effect of pH

The effect of pH on the bioconversion of heptanal is illustrated in Figure 3. The conversion was

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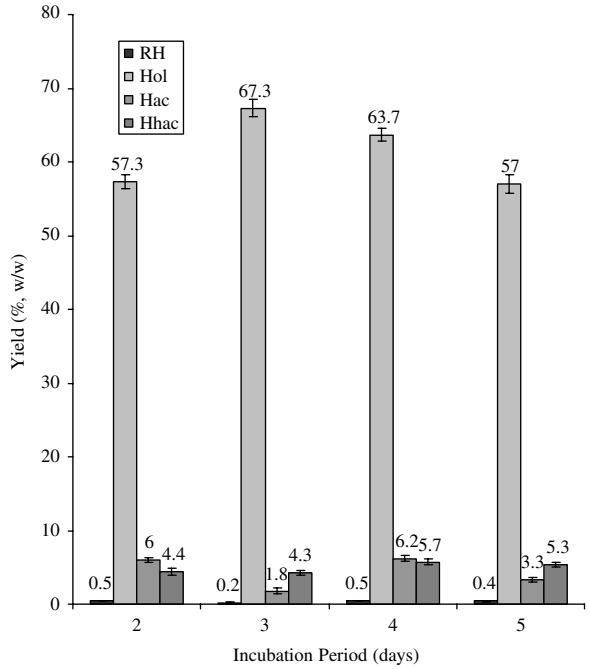

Figure 2. Effect of incubation period on bioconversion of heptanal by *S. cerevisiae* (Sc). RH, residual heptanal; Hol, heptanol; Hac, heptanoic acid; Hhac, 2-hydroxyheptanoic acid

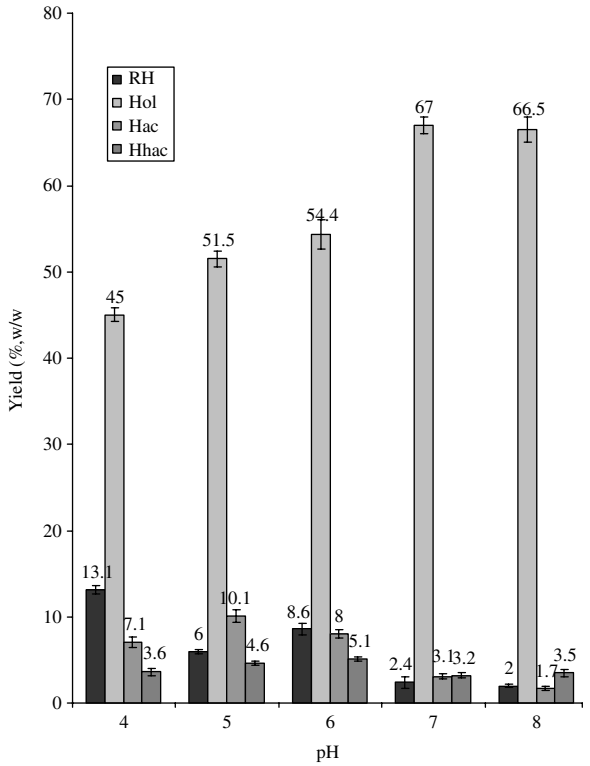

Figure 3. Effect of pH on bioconversion of heptanal by *S. cerevisiae* (Sc). RH, residual heptanal; Hol, heptanol; Hac, heptanoic acid; Hhac, 2-hydroxyheptanoic acid

studied at pH 4.0–8.0. The maximum conversion of heptanal to heptanol ($67.0 \pm 0.98\%$ w/w) took place at pH 7.0. The lowest conversion ($45.0 \pm 0.8\%$ w/w) of heptanal to heptanol occurred at pH 4.0. However, the heptanoic acid content was found to decrease from 3.1 ± 0.3 to $1.7 \pm 0.25\%$ w/w when the pH was changed from 7.0 to 8.0. The yield of 2-hydroxyheptanoic acid was found to be minimal ($3.2 \pm 0.3\%$ w/w) at pH 7.0, whereas it reached its highest ($5.1 \pm 0.25\%$ w/w) level at pH 6.0. It was therefore found that the conversion of heptanal to heptanol increased with decreasing acidity of the medium.

Effect of substrate concentration

The heptanal concentration in the medium is also an important parameter, since excess addition may lead to the presence of residual substrate in the product mixture. Its concentration was therefore also optimized. Figure 4 depicts the effect of substrate concentration on its conversion to heptanol and heptanoic acid. The optimum concentration of heptanal, resulting in maximal conversion (with no residual heptanal) to heptanol ($68.2 \pm 0.85\%$ w/w), was found to be 0.15 ml/100 ml medium. Under this condition it was also observed that the yield of heptanoic acid ($0.6 \pm 0.05\%$ w/w) and 2-hydroxyheptanoic acid ($3.1 \pm 0.2\%$ w/w) was least. The yield of 2-hydroxyheptanoic acid obtained was found to be highest ($9.5 \pm 0.6\%$ w/w) at the substrate concentration of 0.25 ml/100 ml medium. With increasing heptanal concentration in the medium, its conversion to heptanol was observed to decrease ($68.2 \pm 0.85\%$ to $49.0 \pm 1.3\%$ to $30.3 \pm 1.4\%$ w/w), whereas the reverse was found for conversion to heptanoic acid ($0.6 \pm 0.05\%$ to $10.1 \pm 0.4\%$ to $10.5 \pm 0.6\%$ w/w).

Effect of yeast concentration

The concentration of dry yeast used for the bioconversion was also important, since it provided the necessary enzyme system leading to the characteristic change. Under the optimized incubation period, pH and heptanal concentration, the effect of

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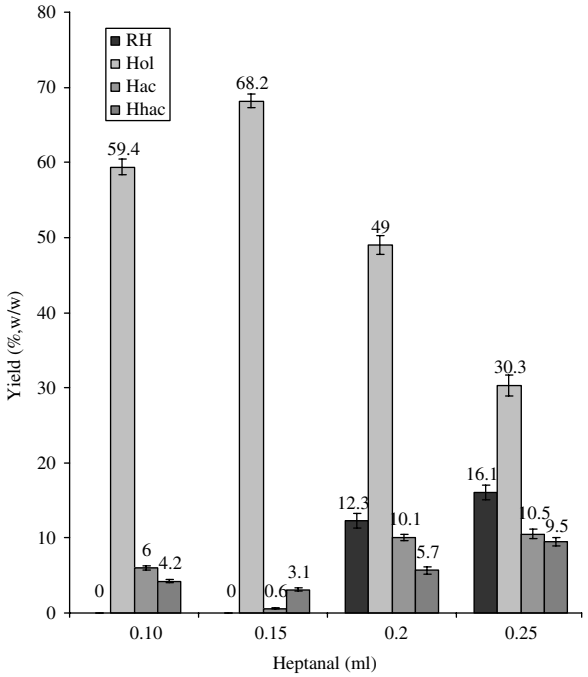

Figure 4. Effect of heptanal concentration on bioconversion by *S. cerevisiae* (Sc). RH, residual heptanal; Hol, heptanol; Hac, heptanoic acid; Hhac, 2-hydroxyheptanoic acid

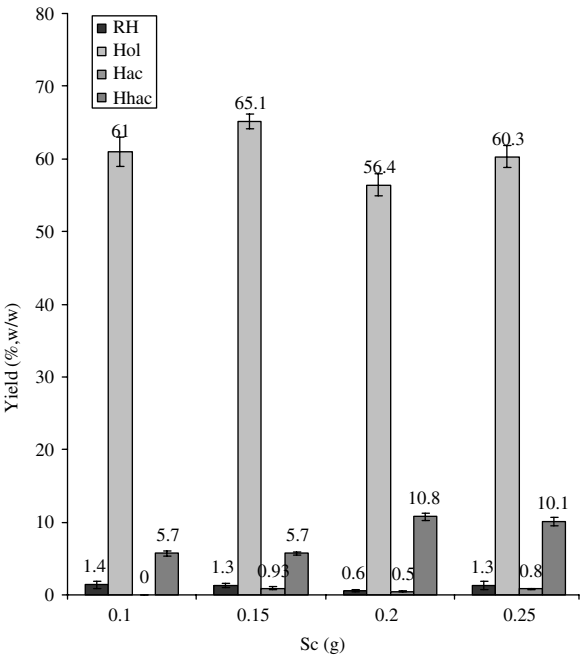

Figure 5. Effect of *S. cerevisiae* (Sc) concentration on bioconversion of heptanal. RH, residual heptanal; Hol, heptanol; Hac, heptanoic acid; Hhac, 2-hydroxyheptanoic acid

variation in dry yeast concentration was therefore studied (see Figure 5). Since the maximum conversion to heptanol ($65.1 \pm 1.0\%$ w/w) and heptanoic acid ($0.93 \pm 0.25\%$ w/w) was found in the medium in which 0.15 g yeast was added, the optimum *S. cerevisiae* concentration was assumed to be 0.15 g/100 ml medium. However, the yield of 2-hydroxyheptanoic acid became highest ($10.8 \pm 0.5\%$ w/w) in medium to which 0.20 g yeast was added.

Effect of variation of maltose concentration

Figure 6 shows the effect of different concentrations of maltose used in the medium. The effects of different parameters for biotransformation were studied, keeping the maltose content at 0.5 g/100 ml medium. However the effect of variation of variation of maltose content in the medium was studied, since maltose and substrate (heptanal) both act as sources of carbon and may compete in its use. The variation in content of maltose showed maximum conversion of heptanal to heptanol ($68.9 \pm 1.1\%$ w/w) in medium containing 0.3 g maltose/100 ml medium, with simultaneous formation of a much smaller amount ($1.3 \pm 0.35\%$ w/w) of 2-hydroxyheptanoic acid. Under this condition the residual heptanal content ($1.1 \pm 0.25\%$ w/w) was also found to be less.

Conclusion

Although the use of *S. cerevisiae* for biotransformation of different substrates is well documented, its efficiency to transform heptanal into other product(s) has not been reported previously. An attempt was therefore made to study the bioconversion of heptanal using *S. cerevisiae*. The investigation revealed that *S. cerevisiae* can effectively transform heptanal to heptanol as the major product with a yield of $68.9 \pm 1.1\%$ w/w under optimum conditions. The other products obtained are heptanoic acid and 2-hydroxyheptanoic acid.

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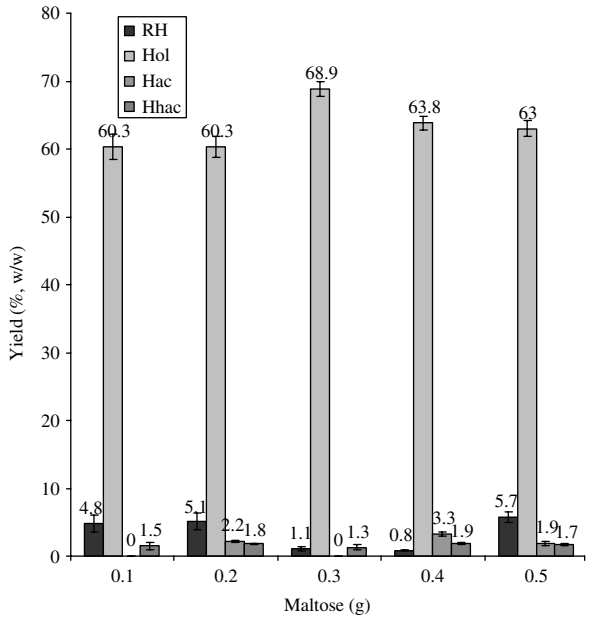

Figure 6. Effect of variation of maltose concentration on bioconversion of heptanal. Sc, *S. cerevisiae*; RH, residual heptanal; Hol, heptanol; Hac, heptanoic acid; Hhac, 2-hydroxyheptanoic acid

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